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Bugs for atopy: the *Lactobacillus rhamnosus* GG strategy for food allergy prevention and treatment in children

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REVIEW ARTICLE

Abstract

Food allergy (FA) is a major health issue for children living in Western countries. At this time the only proven treatment for FA is elimination of offender antigen from the diet. It is becoming clear that the development of gut microbiota exerts a profound influence on immune system maturation and tolerance acquisition. Increasing evidence suggests that perturbations in gut microbiota composition of infants are implicated in the pathogenesis of FA. These findings have unveiled new strategies to prevent and treat FA using probiotics bacteria or bacterial substance to limit T-helper (Th)/Th2 bias, which changes during the disease course. Selected probiotics administered during infancy may have a role in the prevention and treatment of FA. *Lactobacillus rhamnosus* GG (LGG) is the most studied probiotic in this field. Administration of LGG in early life have a role in FA prevention. Preliminary evidence shows that LGG accelerates oral tolerance acquisition in cow's milk allergic infants. We are understanding the mechanisms elicited by LGG and metabolites in influencing food allergen sensitization. A deeper definition of these mechanisms is opening the way to new immunotherapeutics for children affected by FA that can efficiently limit the disease burden.

Keywords: gut microbiota, short chain fatty acids, butyrate, eczema, cow's milk allergy, oral tolerance

1. Introduction

Food allergy (FA) is an increasing public health problem (Berin and Sampson, 2013). Cow's milk allergy (CMA) is one of the most common FA in early childhood, with an estimated prevalence ranging between 2 and 3% in infants (Apps and Beattie, 2009). During the last decade, we observed a changing pattern in FA with increased prevalence, severity of clinical manifestations and risk of persistence until later ages. The Centers for Disease Control and Prevention documented an 18% increase among children in the USA between 1997 and 2007. In the same country, FA accounts for about 30,000 emergency room visits and 150 deaths per year (http://foodallergy.org). Similarly, in Italy we observed that the number of hospital admissions for food-induced anaphylaxis doubled in only 5 years, and that cow milk proteins were the leading food allergens (Berni Canani *et al.*, 2012a). There is evidence that resolution rates have slowed for allergies that have been commonly outgrown, such as those to milk, egg, wheat and soy. For example, Elizur *et al.* (2012) in a population-based study reported that only 57.4% of CMA children resolved their allergy at 5 years of age. FA has deleterious effects on family economics, social interactions, school and work attendance, and health-related quality of life; it can be costly in terms of medical visits and treatments (Sicherer and Sampson, 2014). Given the morbidity resulting from FA, there is considerable interest in generating efficient approaches that may stimulate oral tolerance acquisition and maintenance. Rising disease prevalence over a short period of time cannot be explained by genetic variation alone, renewing interest in the role of the environment in

shaping allergic sensitisation to food. As our knowledge of the crucial influence of gut microbiota on the maturation of immune system has grown, more recent evidences support the idea that alterations of gut microbiota composition induced by environmental factors (e.g. antibiotics, diet, sanitation) may play a central role in the occurrence of FA. At the same time, increasing evidence indicates development of gut microbiota as a crucial factor for immune system maturation and tolerance acquisition (Gourbeyre *et al.*, 2011). These data support the use of probiotics, defined as live microorganism that when consumed in adequate amounts as part of food or as oral supplements confer a health benefit on the host (Hill *et al.*, 2014), as potential preventive and therapeutic strategy for FA. *Lactobacillus rhamnosus* GG (LGG) is the probiotic formulation most often associated with clinical efficacy in FA. In this paper, we discuss the most recent evidences that support the role of probiotics, and in particular of LGG, in the prevention and treatment of FA.

2. Microbiota as potential target for food allergy

The crucial role of gut microbiota in the pathogenesis of FA is supported by several lines of evidence deriving from clinical and basic science studies. The more recent and relevant evidences are the following.

Dysbiosis

Imbalance in intestinal microbiota composition has been documented in patients with FA. Ling *et al.* (2014) showed that several key FA-associated bacterial phylotypes, but not the overall gut microbiota diversity, significantly changed in infants with FA. Nakayama *et al.* (2011) profiled the faecal bacteria compositions in allergic and non-allergic infants using the 16S rRNA gene short-tag pyrosequencing approach and correlated some anomalies in the microbiota with allergy development in later years. The comparative analysis of genus-level composition data identified population differences in some genera between the allergic and non-allergic groups. Interestingly, allergic infants showed high colonisation of *Bacteroides* and/or *Klebsiella* and less colonisation of *Clostridium perfringens/butyricum*, suggesting antagonism between these bacterial groups in the gastro-intestinal tract (Nakayama *et al.*, 2011). This finding is apparently in contrast with previous studies in which *Clostridia* were more abundant in allergic infants (Kalliomaki *et al.*, 2001a,b; Smehilova *et al.*, 2008). This discrepancy may be attributable to species differences, because *Clostridia* contain a wide number of different bacterial strains. Reduced microbial encounter has been hypothesised to play a role in the development of allergies. Caesarean delivery limits the input of maternal bacteria during birth and may thus be a risk factor. A positive association between caesarean section and food allergy has been reported (Eggesbo *et al.*, 2005).

Antibiotics

Maternal use of antibiotics before and during pregnancy, as well as antibiotic courses during the first month of life, are associated with an increased risk of CMA in children (Metsälä *et al.*, 2013). Antibiotic use during infancy potently perturbs intestinal bacteria populations and has often been cited as contributing factor to the rising prevalence of allergic disease (Blaser, 2011).

Diet

In addition to improve hygiene, the nutritional change that has occurred in the Western world over the past few decades coincides with the prevalence of atopic and autoimmune diseases. Interventional studies have shown that high fat, and low fruit and vegetables consumption is linked to worse allergy. A dietary basis for inflammatory diseases is most likely explained by interactions between dietary or bacterial metabolites and immune cells, or pathways for gut homeostasis (Thorburn *et al.*, 2014). An infant diet consisting of high levels of fruits, vegetables, and home-prepared foods is associated with less food allergy by the age of 2 years (Grimshaw *et al.*, 2014).

Animal models

Mice with FA exhibit a specific gut microbiota signature that is able to transmit disease susceptibility and is subject to reprogramming by enforced tolerance (Rivas *et al.*, 2013). The allergy reducing effects of probiotics against food allergens have been demonstrated in murine models of FA. Studies with germ-free mice indicated that the interaction between allergens and the host's gut microbiota plays a crucial role in oral tolerance development and in reducing secretions of allergens specific antibodies. Germ-free animals do not develop oral tolerance and maintain a T helper 2 (Th2) type immune response to oral antigens. This could be correct by the reconstitution of the microbiota at the neonatal stages, but not by reconstitution at later ages (McDermott and Huffnagle, 2014). These findings suggest a crucial role of gut microbiota for oral tolerance acquisition. Atarashi *et al.* (2013) demonstrated that mice gut colonisation with selected 17 *Clostridia* strains stimulates Treg cells expansion and differentiation and induces anti-inflammatory cytokines including interleukin (IL)-10 and transforming growth factor (TGF)-β. Oral inoculation of *Clostridium* during the early life of conventionally reared mice resulted in resistance to colitis and down regulation of systemic immunoglobulin E responses in adult mice, suggesting a new therapeutic approach to allergy (Stefka *et al.*, 2014). Thang *et al.* (2011) investigated the effects of LGG supplementation on mice sensitised with the whole CMP. LGG administration seems to favour suppression of the Th2 response and promotion of Th1 response (Thang *et al.*, 2011).

3. Probiotics for food allergy prevention

Most randomised controlled trials evaluated infants at high risk for allergy, defined as more than one family member having any allergic disease. Most of these studies looked primarily at early outcomes of allergic disease, such as eczema. A large number of clinical studies and meta-analyses have been published on this topic with conflicting results (Dang *et al.*, 2013; Doege *et al.*, 2012; Mugambi *et al.*, 2012; Osborn and Sinn, 2007; Pelucchi *et al.*, 2012). Differences in study design, populations, probiotic strains and dosages are responsible for these discrepancies (Berni Canani and Di Costanzo, 2013b; Castellazzi *et al.*, 2013; Elazab *et al.*, 2013; Ismail *et al.*, 2013; Kim *et al.*, 2013; Lau, 2013). It is evident that different effects may be observed, depending on the strain of the microorganism used (Klaenhammer *et al.*, 2012). Prenatal and postnatal administration of high doses of LGG seems to be the most promising approach (Table 1) in particular on reducing total immunoglobulin E (IgE) and atopic sensitisation (Elazab *et al.*, 2013). Thus, carefully selection of particular probiotic strategy during pregnancy and early infancy is mandatory to obtain positive results and to limit negative outcomes. In fact, it has been demonstrated that the administration of *Lactobacillus acidophilus* is associated with an increased risk of atopic sensitisation (Elazab *et al.*, 2013).

4. Probiotics for food allergy treatment

Administration of LGG to food allergic children (age <2 years, challenge-proven and affected by mild to moderate eczema) improved the atopic eczema score significantly (Majama and Isolauri, 1997). A Cochrane published in 2008 (Boyle *et al.*, 2008), based on analysis of small numbers of participants, suggested that even if probiotics were not an effective treatment for eczema, a significant benefit could not be confidently excluded. Studies in infants with eczema who received formulas supplemented with LGG showed benefits in decreasing gastrointestinal symptoms (Isolauri *et al.*, 2000). For instance, after a challenge study in infants allergic to CMP, faecal IgA levels were detected to be higher, and tumour necrosis factor alpha (TNF-α) levels were lower in the LGG applied group compared to placebo. Moreover, LGG is able to induce interferon-gamma (IFN-γ) secretion in infants with CMA and IgE-associated dermatitis, but not in infants without CMA, suggesting that the pattern of intestinal microbiota may be aberrant in infants with an atopic predisposition, and the beneficial effects of probiotics could be evident only in allergic subjects (Pohjavuori *et al.*, 2004).

The addition of LGG to an extensively hydrolysed casein formula (eHCF) improved the recovery of inflamed colonic mucosa vs eHFC alone in infants with CMA-induced colitis, demonstrated with a decrease in faecal calprotectin and in the number of infants with positive stools occult blood test after 1 month (Baldassarre *et al.*, 2010).

Apart from rapid resolution of symptoms, one of the main objective in FA treatment is tolerance acquisition (Tang and Martino, 2013). We have demonstrated that treatment of CMA infants with an eHFC supplemented with LGG accelerates oral tolerance acquisition (Berni Canani *et al.*, 2012b). Infants (age 1-12 months), consecutively referred for suspected CMA, but still receiving cow's milk proteins, were invited to participate in the study. Subjects were randomly allocated to one of the two groups of dietary intervention: a control group, who received an eHCF; and an active group, who received eHCF containing LGG (at least 1.4×10^7 cfu/100 ml). After 12 months, the double-blind placebo-controlled food challenge was negative in 15 of 28 control infants (53.6%) and in 22 of 27 infants receiving eHCF with LGG (81.5%; P=0.027). In a subsequent study, otherwise healthy infants with CMA receiving eHCF, eHCF with LGG, hydrolysed rice formula, soy formula, or amino acid-based formula, were assessed after 12 months of dietary treatment for possible oral tolerance acquisition by food challenge. The rate of tolerance after 12 months was significantly higher in the groups receiving eHCF (43.6%) or eHCF plus LGG (78.9%) compared with other groups: hydrolysed rice formula (32.6%), soy formula (23.6%), and amino acid-based formula (18.2%) (Berni Canani *et al.*, 2013a). LGG is known to modulate immune functions via various pathways, including those involving enterocytes, monocytes, mast-cells, dendritic cells, and regulatory T cells (De Kivit *et al.*, 2014). Administration of LGG is associated with a complex response in intestinal mucosa, reflected by the up- and down-regulation of several genes involved in the immune response, inflammation, cell-cell signalling, signal transcription and transduction. LGG alters the generation of cytokines that may be involved in IgE- or non-IgE-mediated CMA (i.e. IL-4, IL-5, IL-10, IFN-γ, TGF-β, TNF-α), and thereby can positively modulate the major pathways involved in CMA pathogenesis. These effects depend mainly on the combined activity of different LGG molecules (lipoteichoic acid, secreted proteins, exopolysaccharides, DNA) (Segers and Lebeer, 2014).

It is important to recognise that these results cannot be generalised to other probiotics or other *Lactobacillus* strains. Other *Lactobacillus* strains have different modes of action and varied effectiveness on immune systems. Hol *et al.* (2008) showed that supplementation of a combination of *Lactobacillus casei* CRL431 and *Bifidobacterium lactis* Bb 12 to an extensively hydrolysed CMP formula failed to induce tolerance during 12 months of treatment in infants with CMA. The differences between *Lactobacillus* strains is further demonstrated by comparative genomics studies that reveal that LGG contains 331 strain-specific proteins.

Table 1. Main allergy prevention studies using probiotics.

Investigators • Population / Probiotics and doses	Prenatal administration	Postnatal administration	Allergy preventive effect
Kalliomaki *et al.* (2001a, 2003) • Mothers with ≥1 first-degree relative (or partner) with allergic disease • *Lactobacillus rhamnosus* GG (1×10^{10} cfu/day) (only to mother if breast feeding post-natal)	Yes, 2-4 weeks before delivery	Yes, 6 months (only to baby if not breastfeeding)	Yes, at 2 and 4 years
Rautava *et al.* (2006) • Need for artificial feeding before 2 months of age • *L. rhamnosus* GG (1×10^{10} cfu/day) + *Bifidobacterium lactis* (1×10^{10} cfu/day) added to infant formula	No	Yes, from <2 months (depending on age started formula) until 12 months	No
Taylor *et al.* (2007); Jensen *et al.* (2012) • Mother with positive Skin Prick Test (SPT) or documented allergic disease • *Lactobacillus acidophilus* (3×10^{8} cfu/day)	No	Yes, 6 months direct to infant	No, at 1 and 5 years
Kukkonen *et al.* (2007); Kuitunen *et al.* (2009) • One or both parents with allergic disease • *L. rhamnosus* GG and LC705 (both 5×10^{9} cfu twice daily) + *Bifidobacterium breve* and *Proprionibacterium freudenreichii* (both 2×10^{9} cfu twice daily)	Yes, 2-4 weeks before delivery	Yes, 6 months direct to infant	Yes, at 2 years. No, at 5 years (except decrease in atopic eczema in caesarean-delivered children)
Abrahamsson *et al.* (2007, 2013) • Families with allergic disease • *Lactobacillus reuteri* (1×10^{8} cfu/day)	Yes, 2-4 weeks before delivery	Yes, 12 months direct to infant	No, at 2 and 7 years
Kopp *et al.* (2008) • Pregnant women from families with ≥1 first-degree relative with an atopic disease • *L. rhamnosus* GG (1×10^{10} cfu/day) to mother if breast feeding post-natal for 3 months, than to the neonates for additional 3 months	Yes, 4-6 weeks before delivery	Yes, 6 months direct to infant	No, at 2 years
Wickens *et al.* (2008, 2012) • One or both parents with allergic disease • *L. rhamnosus* HN001 (1×10^{10} cfu/day) or *B. lactis* (1×10^{10} cfu/day) HN019	Yes, 2-5 weeks before delivery	Yes, 2 years to infant regardless of feeding method	Yes, at 2 and 4 years
Huurre *et al.* (2008) • Mother with current atopic disease • *L. rhamnosus* GG + *B. lactis* (both at 1×10^{10} cfu/day)	Yes, from first trimester	Yes, end of exclusive breastfeeding	No
Soh *et al.* (2009); Loo *et al.* (2014) • Any first degree relative with SPT+ allergic disease • *L. rhamnosus* LPR (1×10^{9} cfu/day) + *Bifidobacterium longum* BL999 (6×10^{8} cfu/day)	No	Yes, 6 months in infant formula	No, at 1 and 5 years
Niers *et al.* (2009) • Atopic disease in either mother or father plus at least one sibling • *Lactococcus lactis W58* + *B. lactis W52* + *Bifidobacterium bifidum W23* (each at: 1×10^{9} cfu/day)	Yes, 6 weeks before delivery	Yes, 12 months (direct to infant)	Yes
West *et al.* (2009, 2013) • Atopic disease in either mother, or sibling • *Lactobacillus paracasei* strain F19 (1×10^{8} cfu/day in weaning cereal)	No	Yes, 4-13 months during weaning	Yes, at school-age. No long-term effects (8-9 years)
Dotterud *et al.* (2010) • Unselected population • *L. rhamnosus* GG + *L. acidophilus* LA5 + *B. lactis* Bb-12 (each at 5×10^{10} cfu/day)	Yes, from 36 weeks	No, given to the breastfeeding mother for 3 months	Yes
Kim *et al.* (2010) • Pregnant women with a family history of allergic diseases • *B. bifidum* BGN4 + *B. lactis* AD011 + *L. acidophilus* AD031 (each at 1.6×10^{9} cfu/day) in 0.72 g of maltodextrin and 0.8 g of alpha-corn	Yes, 4-8 weeks before delivery	Yes, 6 months after delivery	Yes, at 1 year

Tabel 1. Continued.

Investigators • Population / Probiotics and doses	Prenatal administration	Postnatal administration	Allergy preventive effect
Boyle *et al.* (2011) • Pregnant women carrying infants at high risk of allergic disease • *L. rhamnosus* GG (1.8×10^{10} cfu/day)	Yes, from 36 weeks gestation until delivery	No	No, at 1 year
Rautava *et al.* (2012) • Mothers with allergic disease and atopic sensitisation • *L. rhamnosus* LPR + *B. longum* BL999 or *L. paracasei* ST11 + *B. longum* BL999 (each at 1×10^{9} cfu/day)	Yes, 2 months before delivery	Yes, 2 months of breast feeding	Yes
Allen *et al.* (2014) • Unselected population • *Lactobacillus salivarius* CUL61 (6.25×10^{9} cfu/day) + *L. paracasei* CUL08 (1.25×10^{9} cfu/day) + *Bifidobacterium animalis* ssp. *lactis* CUL34 (1.25×10^{9} cfu/day) + *B. bifidum* CUL20 (1.25×10^{9} cfu/day)	Yes, from 36 weeks gestation until delivery	Yes, 6 months direct to infant	Yes, at 6 months and 2 years

Finally, it has been recently demonstrated that daily supplement of LGG resulted in a dramatic shift in the composition of the intestinal microbial community with a large increase in the number of taxa previously associated with a decreased risk for the development of allergy and atopy (Cox *et al.*, 2010). We used high throughput sequencing technology (16S rRNA-based sequence analysis) to compare faecal samples from newly diagnosed CMA infants (n=12, 9 male, mean age 4.33 m) before and after treatment with eHCF plus LGG. Treatment with eHCF containing LGG, but not eHCF alone, expanded gut microbiota populations associated with immunoregulatory effects and increased butyrate production at intestinal level. We found a significant positive correlations between faecal butyrate concentration and the abundance of four clostridia genera: *Faecalibacterium*, *Blautia*, *Roseburia*, and *Coprococcus*. All four genera resulted increased in CMA infants after treatment with eHCF plus LGG. A protective role for butyrate was also confirmed in a murine model of CMA. C3H/HeOuJ mice pretreated for 2 weeks with butyrate (20 mg/kg/d) before oral sensitisation with beta-lactoglobulin (20 mg) showed a significant reduction of sIgE and IL-4 production (Berni Canani *et al.*, 2014). Our data suggests that eHCF containing LGG promotes tolerance in infants with CMA, in part, through its influence on the community structure of the gut microbiota, this mechanism acts in combination with the activity of LGG immunoregulatory components (Figure 1). These data support the importance of a 'nutritional immunology approach' able not only to efficiently cure the symptoms, but also to accelerate tolerance acquisition (Cao *et al.*, 2014; Nermes *et al.*, 2013).

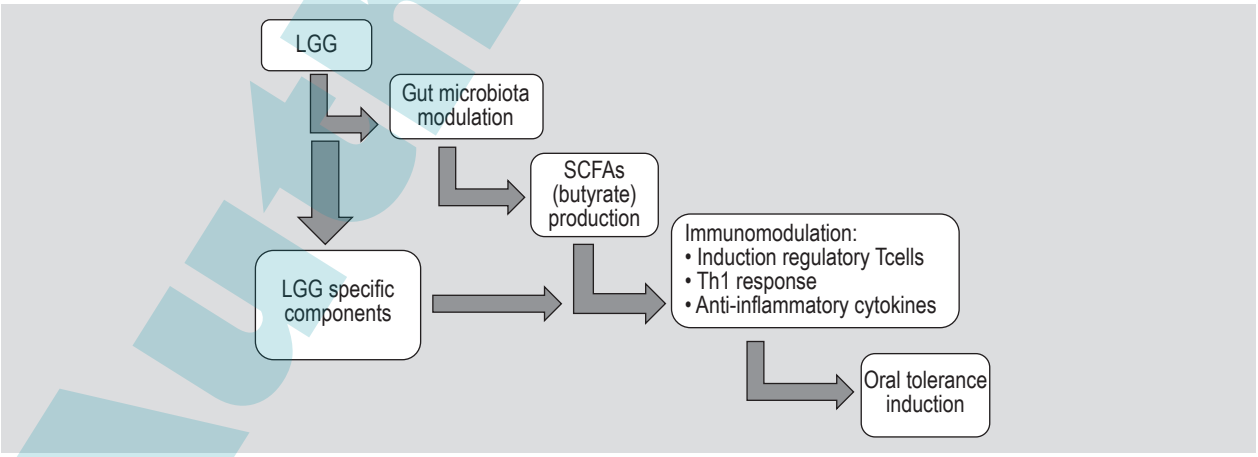

Figure 1. The multiple mechanisms elicited by the probiotic *Lactobacillus rhamnosus* GG (LGG) in inducing oral tolerance acquisition in children affected by food allergy. There is a synergistic effect mediated by immunoregulatory components of *L. rhamnosus* GG, and its efficacy in regulating composition and function of gut microbiota. An increased production of butyrate deriving from the gut microbiota composition shaping is able through a direct interaction with the immune system to stimulate a Th1 response.

5. Conclusions

It is becoming clear that the composition and metabolic activity of the intestinal microbiome exerts a crucial influence on immune development and function. These findings are contributing to a better knowledge in the FA pathogenesis and are opening new preventive and therapeutic strategies. There is an extensive literature documenting the efficacy of LGG for prevention and treatment of food allergy. We are understanding the mechanisms elicited by LGG, and its metabolites, in influencing food allergen sensitisation. A deeper understanding of these mechanisms is opening the way to new immunotherapeutic strategies for children affected by FA that can efficiently limit the disease burden.

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